[細胞の採取方法]

自己末梢血液採取（細胞移植群のみ）

プロトコル治療実施研究機関で約400mLの自己末梢血液採取を行い、血清を分離凍結後、試験物調製施設に輸送する。必要に応じて、1回目の自己血採取の1週間後(細胞培養開始後）にも、同様の手順で2回目の自己血清採取並びに試験物調製施設への輸送を行う。

骨髄液の採取（細胞移植群のみ）

プロトコル治療実施研究機関で定められた手順書に従って約30mLの自己骨髄液の採取を行い、直ちに血液搬送用クーラーボックスに保存して試験物調製施設に搬送する。温度変化はクーラーボックスに設置された温度センサーでチェックして記録する。

細胞、血清の調製（細胞移植群のみ）

試験物調製施設の使用に関する教育訓練を受けたプロトコル治療実施研究機関調製担当者が、試験物調製施設の試験物調製支援担当者と共同にて、施設で定められた手順書に従い、細胞及び血清の調製を行う。培養液に15%自己血清を加える。

[調製方法]

試験物調製施設の手順書に従い、骨髄液約30mLに培養液を加え、T-500フラスコ6個で培養する。培養液は3～4日毎に交換する。培養開始から数日後に接着性の骨髄間葉系細胞が出現する。赤血球等の非接着細胞は培養液交換時に除去される。9～12日後に細胞を剥離して、遠心分離法で回収する。自己血清とヒアルロン酸の混合液に細胞を懸濁し、移植用細胞（試験物）とする。試験物を搬送容器に入れ、試験物調製施設からプロトコル治療実施研究機関に冷蔵輸送する。輸送中の温度変化を輸送容器内に設置された測定装置で記録する。

[移植・投与方法]

搬送された細胞の品質管理成績をチェックし、定められた標準作業手順書に従って調製された細胞であることを確認する。プロトコル治療実施研究機関で、関節鏡手術を施行し関節軟骨欠損部を確認、同部に骨髄刺激法を施行する。

試験物を関節内に注入し、創部を縫合し手術を終了する（標準治療群では、骨髄刺激法の施行のみ)。術後は抗菌薬を投与して感染予防を行なう。

[観察・評価]

中間評価：効果安全性評価委員会により、有害事象の有無、種類、重症度を基に、臨床研究継続の適否の確認

（細胞移植群5例登録後、6週間の観察終了時）

主要評価項目：

IKDC subjective scoreのプロトコル治療前と治療48週後における改善度

副次評価項目：MRI、局所単純X線

Knee injury and Osteoarthritis Outcome Score：KOOS

血清KS値

有害事象

(倫理面への配慮)

本臨床研究は、ヘルシンキ宣言に基づく倫理的原則に留意し、「ヒト幹細胞を用いる臨床研究に関する指針」及び実施計画書を遵守して実施する。

自己細胞移植を受ける患者に対して、試料提供者に一切不利益、危険性が伴わないように配慮し、人権擁護を含めたインフォームドコンセントのもとに施行する。研究目的を含め、研究内容の倫理的、科学的妥当性について適正な倫理委員会等による審査・承認を得た上で施行する。

C．研究結果

１例の軟骨欠損患者に対して本報告書作成時点において移植のための準備を行っている。23歳女性。６年前に半月板切除術を施行されている。数年前から膝痛が出現し当科受診。MRIにて２平方cmの軟骨欠損あり、患者の同意を得て臨床研究の登録を行った。ランダム化によって細胞移植群に分けられ、自己血清採取、骨髄血採取行い、現在培養中である。

D．考察

なし

E．結論

なし

G．研究発表

1．論文発表

なし

2．学会発表

なし

H．知的財産権の出願・登録状況

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

厚生労働科学研究費補助金（再生医療実用化研究事業）
分担研究報告書

関節鏡視下自己骨髄間葉系幹細胞移植による関節軟骨欠損修復
—多施設共同、非盲検、ランダム化、並行比較試験—

研究分担者　名井　陽　大阪大学医学部附属病院　准教授

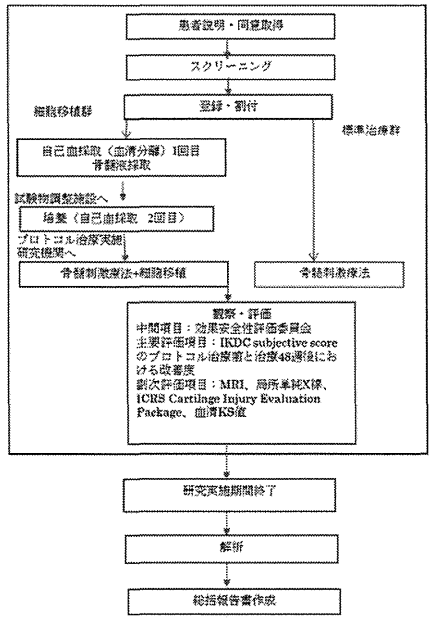
研究要旨
大阪市立大学および兵庫医科大学のヒト幹細胞臨床研究について、各施設より１例ずつ被験者の細胞培養を受託し、各施設で細胞治療を行うことができた。また、本研究の調整事務局として再生医療等の安全性の確保等に関する法律への対応・移行の準備を行い、細胞培養加工施設の届出は本年度内に完了する。再生医療等実施届については、次年度５月を目途に対応を完了するよう資料作成等を進めている。

A．研究目的

細胞移植による関節軟骨損傷修復法は、我が国でもようやく製造販売承認されたところであるが、現在の方法はまだ満足のできる方法とはいえない。本研究では、従来の方法に比べてより手術侵襲の小さい、関節を切開せずに関節鏡視下手術で自家骨髄由来間葉系幹細胞を移植する方法の開発を実施している。これまで、前臨床試験では有効性・安全性が証明されているが、今回、ヒトの関節軟骨修復での有効性・安全性について評価を行うため、広島大学、兵庫医科大学、奈良医科大学、大阪市立大学、近畿大学を含む西日本を中心とした病院において、試験群40例、対照群40例の非盲検、ランダム化、並行比較試験を多施設共同研究として計画した。このうち大阪市立大学、兵庫医科大学にはヒト移植用細胞培養施設（CPC）がない。これらの病院の患者の骨髄液および末梢血を大阪大学未来医療センターに送り、培養増殖させ、各施設に送り返し移植を行う細胞培養依託システムの構築をめざす。

B．研究方法

以下の様なスケジュールで臨床試験を実施する。



大阪市立大学と兵庫医大の被験者の細胞は、以下の手順で大阪大学医学部附属病院未来医療センター細胞培養調整施設にて培養されて製剤化されたあと各大学病院で移植される。

1．大阪市立大学、兵庫医大では末梢血液採取（外来）を行い血清を分離凍結後、大阪大学医学部附属病院未来医療センター（以下、大阪大学未来医療センターと略）のCPCに輸送する。
2．大阪市立大学あるいは兵庫医科大学外来手術室で採取した骨髄液は、直ちに血液搬送用クーラーボックスに保存し大阪大学未来医療センターのCPCに移送する。CPCまでの搬送時間は約60分である。
3．CPC内のアイソレーターで細胞および血清の調製を行う。培養液に15%自己血清をいれる。運び込まれた骨髄液約30mLに培養液を加え20枚のT-75フラスコで培養する（1.5ml/flask)。3日ごとに培養液を交換する、約3日後に接着細胞が出現する。赤血球等の非接着細胞は培養液交換の時に除去される。約10日後、培養細胞が50%コンフルエントに達したところで継代培養する（5×10^5 cells/flaskでT-75フラスコに播種)。約10日後、細胞がほぼコンフルエントに達したら細胞を剥離し、ヒアルロン酸を加えて細胞浮遊液とする。
4．調製細胞を搬送用クーラーボックスに入れて大阪大学未来医療センターCPCから大阪市立大学あるいは兵庫医科大学医学部附属病院に移送する。移送交通手段は公共交通とする。
5．搬送された細胞の品質管理成績をチェックし、GMP基準下で調製された細胞であることを確認する。
6．関節鏡手術を施行し関節軟骨欠損部を確認、同部に骨髄刺激法を施行する。細胞浮遊液を関節内に注入し、創部を縫合し手術を終了する。術後は抗菌薬を投与して感染予防を行う。

（倫理面への配慮）

臨床研究実施計画書、被験者説明文書、同意書、大阪大学医学部附属病院を含む各施設の倫理審査委員会で本研究の承認済みで、4病院とも厚労省「ヒト幹細胞を用いる臨床研究に関する指針」に申請の書類を提出済みである。同指針に関する大臣確認を受ける。

C．研究結果

臨床研究実施の前に、試験物の製造に関して実際のヒト骨髄細胞を利用したコールドランを実施し、一部手順の見直し等を行った。また、改訂されたSOPは大阪大学以外の各研究分担者の細胞製品の製造担当者にも配布し、各施設のハードウエアや施設SOPに即したSOPの修正のための雛形ないしは参考としていただいた。

上記のCPCの対応、及びモニタリング・データマネジメントの体制を確認の上、被験者のエントリーを開始した。大阪市立大学から1例、兵庫医科大学から1例、細胞培養を行った。

臨床研究の実施と並行して、再生医療等の安全性の確保等に関する法律への対応・移行の準備を進めている。CPCは3月中に特定細胞加工物製造施設の届出を行う目途が立った。臨床研究のプロトコルについては、次年度5月を目途に申請する予定である。

D．考察

本研究はヒト幹細胞等の臨床研究に関する指針で実施が認められている、多施設共

同研究に於いて他の医療機関で製造された細胞製剤を臨床研究に使用することを実践する研究である。大阪大学はCPCを持たない大阪市立大学病院と兵庫医科大学病院の被験者の細胞の培養を担当することに加え、本研究全体の調整事務局の役割を担っている。

本年度は、被験者のエントリーが開始され、２施設より２例の細胞培養を請け負い、CPCのない施設の細胞治療を行うことができた。また、再生医療等の安全性の確保等に関する法律への対応の準備として、必要な文書の作成、倫理的手続き等を進めており、次年度５月ごろに対応が完了する予定である。

本研究は、シーズがあってもCPCのない施設の細胞治療を可能にする前例となり、また十分に運用されていない施設の活用促進にもつながり、ひいては再生医療の促進になると考えられる。

E．結論

「関節鏡視下自己骨髄間葉系幹細胞移植による関節軟骨欠損修復―多施設共同、非盲検、ランダム化、並行比較試験」に関して、２施設から２例の細胞培養を請け負った。また、調整事務局として再生医療等の安全性の確保等に関する法律への対応を進めている。

G．研究発表

なし

H．知的財産権の出願・登録状況

なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yamasaki S, Mera H, Itokazu M, Hashimoto Y, Wakitani S.	Cartilage repair with autologous bone marrow mesenchymal stem cell transplantation: review of preclinical and clinical studies.	Cartilage	Vol.5 (4)	196-202	2014
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Article

Cartilage Repair With Autologous Bone Marrow Mesenchymal Stem Cell Transplantation: Review of Preclinical and Clinical Studies

Cartilage
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Abstract

Clinical trials of various procedures, including bone marrow stimulation, mosaicplasty, and autologous chondrocyte implantation, have been explored to treat articular cartilage defects. However, all of them have some demerits. We focused on autologous culture-expanded bone marrow mesenchymal stem cells (BMSC), which can proliferate without losing their capacity for differentiation. First, we transplanted BMSC into the defective articular cartilage of rabbit and succeeded in regenerating osteochondral tissue. We then applied this transplantation in humans. Our previous reports showed that treatment with BMSC relieves the clinical symptoms of chondral defects in the knee and elbow joint. We investigated the efficacy of BMSC for osteoarthritic knee treated with high tibial osteotomy, by comparing 12 BMSC-transplanted patients with 12 cell-free patients. At 16-month follow-up, although the difference in clinical improvement between both groups was not significant, the arthroscopic and histological grading score was better in the cell-transplanted group. At the over 10-year follow-up, Hospital for Special Surgery knee scores improved to 76 and 73 in the BMSC-transplanted and cell-free groups, respectively, which were better than preoperative scores. Additionally, neither tumors nor infections were observed in all patients, and in the clinical study, we have never observed hypertrophy of repaired tissue, thereby guaranteeing the clinical safety of this therapy. Although we have never observed calcification above the tidemark in rabbit model and human histologically, the repair cartilage was not completely hyaline cartilage. To elucidate the optimum conditions for cell therapy, other stem cells, culture conditions, growth factors, and gene transfection methods should be explored.

Keywords

cartilage, bone marrow mesenchymal stem cell, cell transplantation, chondral defect, tissue engineering

Introduction

Articular cartilage covers the ends of bones that form diarthrodial joints, and works as a lubricant and shock absorber. Histologically, articular cartilage is a subset of hyaline cartilage tissue where the extracellular matrix exhibits a collapsed structure lacking blood, lymphatic, or nerve supply, and therefore has poor repair potential. In general, cartilage defects are hardly repaired if they do not penetrate subchondral bone (partial-thickness defects), but could be repaired along with heterogeneous tissue, from fibrous tissue to fibrocartilage, when penetrating subchondral bone (full-thickness defects). However, the reparative tissue, even if appearing as hyaline cartilage histologically, would lack the biochemical capability to express some cartilage-specific molecules, and its biomechanical durability is substantially inferior to that of age-matched normal articular cartilage.[1]

Regarding the prognosis of damaged articular cartilage, defects have not been considered a major problem among many clinicians because they cause few clinical symptoms, at least in the short term. Recently, however, reports have revealed that clinical symptoms or radiological changes

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caused by articular cartilage defects are getting worse when observed for more than 10 years.[2,3] Thus now, articular cartilage defects are thought to need repairing to prevent subsequent osteoarthritis (OA) progression. In fact, articular cartilage defects are indeed a major clinical problem.

To date, we are only partially happy with the repair of articular cartilage defects using current clinical procedures. These procedures usually involves a bone marrow stimulation technique, in which subchondral bone is broken to facilitate cartilage repair from bone marrow–derived cells and growth factors, and consists of multiple perforations,[4] abrasions,[5] and micro-fractures.[6] However, with this procedure, cartilage defects are most often repaired with fibrocartilage, which is biochemically and biomechanically different from normal hyaline cartilage, and this tissue subsequently undergoes degeneration.[1]

Recent studies report the benefits of autologous chondrocyte implantation (ACI)[7] and mosaicplasty.[8,9] Although we can repair small articular cartilage defects using these techniques, their effectiveness is still disputed. Even after ACI and mosaicplasty, some defects continue to persist in the articular cartilage, albeit not in the main weightbearing portions of the joint. In ACI, there is only weak evidence to show the effectiveness[10] and we are forced to sacrifice normal cartilage tissue for harvest, and so an alternative method to obtain autologous cells is preferable.

Further investigations into the repair of articular cartilage defects, using certain other types of cells, have been performed worldwide. Osteochondral progenitor cells or mesenchymal stem cells exist in many kinds of tissue, such as bone marrow, synovium, muscle, and fat (adipose). Autologous cells of these tissues are easily obtained. Within these cells, synovial cells have the best capacity for chondrogenesis, and are a promising source of cells for clinical application.[11] Adipose-derived mesenchymal stem cells are also a noteworthy source for cell therapy.[12,13]

We focused on bone marrow–derived mesenchymal stem cells (BMSC) as a cell source, to explore a new method of cartilage regeneration by studying transplantation of autologous BMSC for articular cartilage defects. Here, we review our work on autologous BMSC transplantation into animals and humans, and show the long-term follow-up outcomes of autologous BMSC transplantation for patients with unicompartmental osteoarthritis containing articular defects in femoral condyle.

Bone Marrow Mesenchymal Stem Cells

In 1966, adherent cells in bone marrow were transplanted into cutaneously formed osteochondral tissue.[14] Since then, cells isolated from postnatal mammalian bone marrow have been shown to have the potential to differentiate into specific cells of mesenchymal tissue, such as bone and cartilage, when implanted in vivo.[15,16] Thus, adherent cells in bone marrow blood contain progenitor cells for bone and/or cartilage. We assumed that these cells were suitable to repair osteochondral joint defects because they could differentiate into both bone and cartilage. We therefore performed autologous culture-expanded BMSC transplantation in a rabbit model.[17]

Preclinical Study

In our rabbit osteochondral defect model, we first collected autologous osteochondral progenitor cells from bone marrow. Next, they were culture expanded and embedded into a collagen gel. These cellular grafts were then transplanted into large (3 mm × 6 mm × 3 mm) full-thickness defects in the weightbearing articular surfaces of 68 rabbits. These transplants were then observed for up to 6 months after surgery.

As early as 2 weeks after transplantation, the defect was mostly replaced with cartilage. The replacement of this repaired cartilage began in the deeper portion of the defect with vascularized bone. By 4 weeks after transplantation, the deeper portion of the defect was almost completely replaced with bone, and 24 weeks after transplantation, subchondral bone was completely repaired without loss or alteration of the overlying articular cartilage. We assume that BMSC preparations rapidly and quantitatively differentiate into chondrocytes in the rabbit distal medial femoral chondyle defect, as has been observed in subcutaneous implantation samples. We hypothesize that these donor chondrocytes and the cartilage tissue that they form, are replaced by host-derived vascular and bone-forming cells up to the bone articular cartilage junction.

We confirmed the effectiveness of BMSC transplantation in the repair of osteochondral joint defects in a rabbit model. Next, we explored whether this technique could be applied in humans. BMSC have a number of suitable properties. First, it is easy to obtain autologous cells. This can be achieved by the aspiration of blood from bone marrow using local anesthesia, without major side effects. Second, we can cause these cells to proliferate without losing their capacity for differentiation, which can then be applied to large articular cartilage defects.

Repair of Articular Cartilage Defects in Humans

All our clinical studies were performed in accordance with the ethical standards of our hospital committee on human experimentation. All subjects enrolled in these studies gave their informed consent, as approved by the institutional committees on human research, who also found these protocols to be acceptable.

In applying BMSC transplantation to chondral defects in humans, the same cell preparation was performed. Briefly, after aspiration of bone marrow blood from iliac crest, nucleated cells were cultured. When the attached cells had reached subconfluence, they were subcultured to expand in culture. Adherent cells were subsequently collected, embedded in a collagen gel, transplanted into the articular cartilage defect in patellae, and covered with autologous periosteum.

Patellar Case Report (First in Humans)

Two patients presented to our clinic because their knee pain prevented them from walking normally.[18] The first case was a 26-year-old woman and the second was a 42-year-old man. After thorough examination, we concluded that the knee pain was due to injured articular cartilage, because there was no other abnormality in their knees. There were no improvements in clinical symptoms despite conservative treatment for several months, so we decided to repair the defect with BMSC transplantation. Three weeks before transplantation, bone marrow was aspirated from the iliac crest of each patient. The cultured cells were subsequently collected, embedded in a collagen gel, transplanted into the articular cartilage defect in the patella, and covered with autologous periosteum. As early as 2 months after transplantation in the first case, we performed arthroscopy and biopsy and found that the defects were covered with tissue showing slight metachromatic staining. Six months after transplantation, clinical symptoms (pain and walking disability) improved considerably, and the improvement persisted for 9 years posttransplantation in one case, and 7 years in the other (at the time of report preparation); both patients are satisfied with the outcome. Two years after the first and 1 year after the second transplantation, arthroscopy revealed that the defects had been repaired with fibrocartilage. We confirmed that autologous BMSC transplantation had been an effective approach for promoting the repair of articular cartilage defects. Now, 16 years following transplantation in the first case and 14 years in the second case, no clinical problem has been reported.

Patellofemoral Joints

In addition, we reported BMSC transplantation into osteochondral defects in 5 knees (femur and patellae) from 3 patients. A 31-year-old woman (bilateral knees), a 46-year-old man, and a 42-year-old man (bilateral knees), underwent BMSC transplantation in their patellofemoral joints. All patients had suffered from pain and clicking in their patellofemoral joints on motion. Because magnetic resonance imaging (MRI) revealed articular cartilage abnormalities in the patellofemoral joints, we performed arthroscopy to confirm the lesions, followed by autologous BMSC transplantation another day. In these cases, we found articular cartilage damage in both the femur and patellae. We removed the damaged articular cartilage, transplanted BMSC embedded in the collagen gel, and covered the transplanted tissue with autologous periosteum. Clinical symptoms improved in all patients.[19]

Femoral Condyle

Kuroda *et al.*[20] reported that transplantation of BMSC into a 20- to 30-mm, full-thickness articular cartilage repair defect in the weightbearing area of the medial femoral condyle of a 31-year-old judo player was effective.[20]

Elbow

We applied this technique to repair osteochondral defects in 3 elbows (humeral capitellum) on three 14-year-old boys.[21] All patients were throwing-athletes and had been suffering from elbow pain during throwing motion. Range of motion was slightly restricted. In radiographs, separated bone fragment was observed in capitellum and diagnosed osteochondral dissecans. Because the separated fragment was large, unstable, and divided into small pieces, meaning it is impossible to reattach this fragment, and we decided to remove the fragment and to transplant autologous BMSC. Clinical symptoms after surgery were much improved in all patients.

Safety of Autologous BMSC Transplantation in Humans

The transformation of cultured cells is a major problem in cell therapy. We have never observed tumor formation in any of our numerous animal experiments or in clinical cases of BMSC transplantation. Although the possibility cannot be excluded, human somatic cells have limited capacity for cell division, and the transformation of cultured adult human BMSC is considered to be rare.

To confirm the safety of BMSC transplantation, we investigated records of all 41 patients who together had received 45 transplantations, including cases mentioned above between January 1998 and November 2008 until their last visit to clinic. Neither tumors nor infections were observed between 5 and 137 (mean of 75) months of follow-up. Therefore we conclude that autologous BMSC transplantation is safe.[22]

Comparative Study for Patients With Knee Osteoarthritis

Results of Our Previous Report

In order to apply this technique to the repair of articular cartilage defects in human osteoarthritic knees, we

transplanted culture-expanded autologous BMSC into the cartilage defects of osteoarthritic knee joints when patients were undergoing high tibial osteotomy (HTO). We then observed the repair tissue at second-look arthroscopic exams when patients were undergoing surgery for removal of the Steinmann pins and staples that fixed the separated proximal tibia.[23] Twenty-four patients with knee OA who underwent HTO were included in this study. Fifteen were female and 9 were male. The patients' average age was 63 years (range 49-70 years). Twelve received autologous bone marrow cell transplants, and 12 were cell-free controls. BMSC were prepared in the same manner. The mean transplanted cell number was 1.3×10^7. HTO was performed using dome osteotomy, fixed with 2 pins with a Charnley clamp and 2 staples. At the time of HTO for OA of the knee, we transplanted these cells embedded in collagen gel into the medial femoral condyle, where articular cartilage was lost and subchondral bone eburnation was exposed. We abraded the eburnated subchondral bone, transplanted cells with collagen, and covered the lesion with autologous periosteum harvested from the anteromedial surface of the tibia. The mean size of the abraded area was 14 mm × 35 mm. The mean follow-up period was 16 months. Before and after surgery, all patients rated their pain (30 points), function (22 points), range of motion (18 points), muscle strength (10 points), flexion deformity (10 points), and instability (10 points), using the Hospital for Special Surgery knee-rating scale.[24]

For the cell-transplanted group, the mean total score was 65.0 points before surgery and 81.3 after surgery, which was significantly improved. For the cell-free group, the mean total score was 66.3 before surgery and 79.2 after surgery, which was also significantly improved. Although the difference in clinical improvement between the groups was not significant, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group. As early as 6.3 weeks after transplantation, defects were covered with white soft tissue, in which metachromasia was partially observed, and 42 weeks after transplantation, the defects were covered with white soft tissue that was much harder than that observed at 6.3 weeks, but was still softer than the surrounding normal cartilage. In most areas of the repair tissue, metachromasia was observed, and the tissue appeared similar to hyaline cartilage.

Long-Term Results of the Comparative Study

We analyzed the clinical results 64 months after transplantation. We could follow 9 out of 12 cell-transplanted patients and 8 out of 12 control patients. As one patient in each group had received total knee replacement, we followed the remaining 8 cell-transplanted and 7 control patients. The mean clinical scores (standard deviation) of the cell-transplanted group and cell-free group were 74 (14) and 76 (16), respectively, which is not a significant difference. Both scores were lower than those of the first report but higher than those before surgery.

Recently, we investigated the long-term clinical results of these patients. At final follow-up, 7 cell-transplanted patients and 7 control patients were available for review. The final follow-up period was 120 and 130 months, respectively. One patient in the cell-transplanted group received total knee arthroplasty. Final HSS scores of the remaining 7 patients in the cell-transplanted group and 7 patients in the cell-free group were 76 (19) and 73 (11), respectively. This may be because some patients were suffering age-related cerebral infarction or femoral neck fracture, and having reduced activity. However, the knee function of these patients was comparable to that at short-term follow-up.

Discussion of BMSC Transplantation

As the clinical symptoms of most patients were improved by autologous culture-expanded BMSC transplantation, this procedure would appear to be effective in the repair of articular cartilage defects, although no direct evidence is available. In this comparative study, the difference in clinical improvement between BMSC-transplanted and control groups was not significant 10 years after the transplantation. HTO itself was effective enough to explain why there was no significant difference for this period. Although the difference in clinical improvement between both groups was not significant, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group. This repair was found to occur much earlier and was better than reported in HTO only or HTO with abrasion.[25,26] Moreover, we want to emphasize that the untreated tibial articular cartilage defects were not repaired with hyaline cartilage at all.

As we showed in the animal experiment, BMSC became chondrocytes and replaced by the host bone under the tidemark. We want to stress that we have never observed calcification above the tide mark in rabbit model. In the original cartilage area, cartilage was formed and in the original bone area, bone was formed. Although we do not know the mechanism, calcification or replacement by bone does not occur above the tidemark. When BMSC are implanted into a weight bearing region, they respond to the mechanical environment in an appropriate way.

In the clinical study, we have never observed hypertrophy of repaired tissue. In some cases where we performed biopsy or MRI, we have never observed calcification above the tidemark in humans, just like in rabbit model.

We also showed that autologous BMSC transplantation is a safe procedure because neither tumors nor infections were observed between 5 and 137 months (mean 75 months) of follow-up.

The important advantage of the technique described here is clear from the data provided. Although these progenitor cells are not abundant, we have been able to mitotically expand them in culture. These approaches have considerable relevance to the treatment of human cartilage defects, and provide the starting point for refinement of a repair technology that is capable, in principle, of regenerating large areas of articular cartilage.

It has been reported that cells isolated from human bone marrow aspirates could be induced to differentiate into other mesenchymal lineages, such as adipocyte, chondrocyte, or osteocyte, in vitro.[27,28] These cells were therefore called mesenchymal stem cells. Furthermore, they also differentiate into cells other than mesenchymal, ectodermal (neurocyte)[29] and endodermal (hepatocyte)[30] tissues (transdifferentiation). Recently, these cells have been considered as a useful source to repair some kinds of tissues, such as bone, cartilage, tendon, muscle, heart, small vessel, liver, nerve, and others.

The number of reports of BMSC transplantation in human articular cartilage is limited. Beside the reports described here, we have found reports from scientific meetings elsewhere in the world. Nejadnik *et al.*[31] reported BMSC transplantation into 36 articular cartilage defects and followed up for 24 months comparing the results with those of 36 ACI. They concluded that BMSC transplantation showed results comparable to ACI, and that it was a good procedure because it required one less step of surgery, reduced costs for patients, and minimized donor site morbidity. However, there are far fewer reports of BMSC transplantations than those of ACI. This is because ACI was explored first and made available for clinical approval very early on by some developed countries. Even in ACI, evidence of effectiveness compared with other procedure is still controversial.[32-34] A long-term follow-up clinical trial with high statistical power is needed to verify the safety and efficacy of new cartilage joint therapy. To date, only the randomized controlled trial in BMSC transplantation compared with ACI, mentioned above, has been reported. This report showed that the clinical effectiveness of BMSC transplantation is comparable to the results of ACI, while BMSC transplantation had superiority in some procedures.

Agung *et al.*[35] reported the BMSC via intra-articular injection was mobilized into the osteochondral defect in the knee joint to explore less invasive procedure than ACI. Wong *et al.*[36] compared HTO combined with injectable BMSC to HTO alone in human osteoarthritic knees. According to this report, the cell-recipient group was superior to the cell-free group in MRI examinations and some clinical evaluations.[36] Thus, the injectable BMSC procedure is effective for osteoarthritis. However, even now, further long-term follow-up studies with high statistical power are needed to establish more evidence.

Other options using cell therapy for cartilage repair are explored at the experimental level. To date, repairable cartilage tissues have not been completely composed of hyaline cartilage histologically, even with the cell combination therapy. Theoretically, hyaline cartilage is preferable with respect to mechanical properties related to durability. Mesenchymal stem cells could be driven into the chondrogenic lineage using cytokine[37-39] or gene transfection,[40,41] and the resulting artificial autogenetic chondrocytes would be transplanted into cartilage defects for improved outcomes. Allogeneic cell transplantation has also been explored in animal models. We have reported that cartilage-like tissue, generated ectopically by muscle-derived cells in a diffusion chamber using bone morphogenetic protein-2, is effective in repairing articular cartilage defects in rats.[39] We also reported that effectiveness of articular cartilage repair using cartilage-like tissue, generated ectopically by amnion-derived cells with bone morphogenetic protein-2.[42] These methods may constitute a new technique of tissue engineering for the repair of articular cartilage defects. Embryonic stem (ES) cells or inducible pluripotent stem cells are the most promising cell sources for many kinds of tissue repair. These cells should also be applicable to the repair of osteochondral defects; however, it is difficult to induce these cells exclusively into chondrocytes. When we transplanted ES cells into joint spaces, they formed a teratoma and subsequently destroyed the joint.[43] However, we have also reported that when transplanted into osteochondral defects, ES cells form cartilage and promote the repair process.[44] The mechanism of this phenomenon is unclear and the use of ES cells might be expected in the future. We also reported that when we transplanted ES cells into an osteochondral defect and fixed the joint by a pin, they formed a teratoma, while cell transplantation without fixation did not induce teratoma but did induce cartilage repair.[45]

Conclusion

Various procedures, including bone marrow stimulation, mosaicplasty, and ACI, have been clinically tried to assess their effectiveness in repairing articular cartilage defects. However, all of them have some demerits. Progenitor cells can proliferate without losing their capacity for differentiation, and we have used this property by transplanting autologous culture-expanded BMSC into articular cartilage defects in human. Our previous reports showed that BMSC treatment improved clinical symptoms, and the safety of this therapy was guaranteed in clinical use. Furthermore, in the clinical study, we never observed hypertrophy of repaired tissue. Although we have never observed calcification above the tidemark in the rabbit model and in humans histologically, the repair cartilage was not completely hyaline cartilage. To elucidate the optimum conditions for cell therapy, different culture conditions, mechanical stresses,

growth factors, and gene transfection methods have been explored, but none of these approaches have been applied clinically. In the future, less invasive administration such as intra-articular injection will be explored and less invasive and more accurate evaluation of cartilage damage will be required.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study was approved by our institutional review board.

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第86回日本整形外科学会学術総会 シンポジウム

「運動器再生医療の最先端」序文

大串 始[1] 脇谷滋之[2]

わが国における再生医療の橋渡し研究の困難さが指摘されて久しい．以前よりアカデミアから国に対し再生医療の促進のための規制緩和の要請が行われ，2011年3月，厚労省医政局長の通達で「アカデミア間での細胞培養委託」が認められたが，それ以外にあまり大きな進展はなかった．2012年，山中伸弥博士によるノーベル賞受賞，安倍政権の成立により，国も再生医療の促進に本腰を入れるようになった．このような状況のもと，2013年4月には再生医療推進法が成立したが，これは「再生医療を進めましょう」という総論的な法律である．さらに踏み込んだ再生医療新法は，「自由診療分野の治療法も含み，安全性により3つに分類してそれぞれ異なる承認の過程を経る」というもので，安全性の高い治療法は承認されやすくなる．画期的なのはこれまで野放し状態で，さまざまな問題を起こしてきた自由診療のもとに行われる治療も規制されることである．また，薬事法を改正し「条件付き承認を認める」ことで速やかに承認を行うべく承認制度を改定することも提言されている．これらの法律により再生医療は推進されると考えられるが，この2つ(再生医療新法と薬事法改正)は2013年1月招集の第183回通常国会での成立困難との判断から取り下げられ，次回国会での成立を目指すことになる．

整形外科領域の再生医療に関しては，関節軟骨修復用の自己軟骨細胞の製造販売が昨年の7月にわが国では初めて承認され，2013年4月保険収載された．これは整形外科領域ではわが国最初の再生医療細胞治療用の商品であるが，実は全体の再生医療を見ても，皮膚細胞に続くわが国では2番目の再生細胞治療の商品である．また，この自家軟骨細胞を使用するにあたっては，その有効性と安全性を十分に理解し，かつ軟骨修復に関する十分な知識・経験を有する整形外科医および施設において軟骨再生を行うことが肝要である．これらに関して日本整形外科学会ではワーキンググループを立ち上げ(松末吉隆委員長)，実施医ならびに施設の基準を策定し，日本整形外科学会としても再生医療の推進に取り組んでいる[1),2)]．

運動器再生医療の臨床研究として，骨，軟骨，椎間板，脊髄，筋肉などの修復のための前臨床研究あるいは臨床研究がわが国では1980年代から行われている．骨に関しては，大串らが奈良県立医大で1980年代から自己骨髄間葉系幹細胞を使った前臨床研究を行い，2001年から同医大の患者骨髄からの間葉系幹細胞の培養を産業技術総合研究所で行い，その培養細胞を用いた臨床研究を行っている．また，大阪大学でも培養を行う専用施設(未来医療センター)を立ち上げ臨床研究が行われている．本シンポジウムでは大串が，自己のみならず同種骨髄間葉系幹細胞移植による骨修復について解説した．軟骨に関しては1990年代後半から島根医大，のちに広島大学に移られた越智光夫先生らによる自己軟骨細胞移植，脇谷らによる自己骨髄間葉系細胞移植の臨床研

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究が行われ，前者は上述のように商品化された．2000年代から，東京医科歯科大学の関矢一郎先生による自己滑膜細胞移植，2010年代からは東海大学の佐藤正人先生による自己軟骨細胞シート移植，大阪大学の中村憲正先生らによる自己滑膜細胞シート移植の臨床研究が行われてきた．本シンポジウムでは関矢先生にご自身の研究についてご解説いただいた．椎間板再生に関しては東海大学の持田譲治先生により自己椎間板細胞移植の基礎研究が開始され，最近，臨床研究が終了したところである．持田先生の共同研究者である酒井大輔先生にご解説いただいた．脊髄損傷に関しては，神経前駆細胞移植からiPS細胞移植，さらにはさまざまな因子投与による前臨床研究が行われてきたが，顆粒球コロニー刺激因子を用いた急性脊髄損傷に対する臨床医師主導型自主臨床試験について千葉大学(現筑波大学)の山崎正志先生にご解説いただいた．筋肉に，細胞移植あるいはさまざまな因子投与による前臨床研究が行われてきたが，広島大学の亀井直輔先生にヒト末梢血CD133陽性細胞，あるいはマイクロRNAによる筋肉修復の前臨床試験についてご解説いただいた．これらの運動器再生医療の橋渡し研究，すなわち前臨床試験から臨床試験，それに続く実用化において，どのような規制があり，何が問題か，それをどのように規制当局が手助けしてくれるか，さらには今回の法改正において何がどのように変わるか，われわれ臨床医が橋渡し研究をスムースに進めるためにはどうすればよいかを，医薬品医療機器総合機構に所属し，しかも整形外科医である岡田潔先生にご解説いただいた．

これらの講演内容を参考に，これから運動器再生医療の橋渡し研究をしようとされている整形外科医のみならず関連する企業の研究者の手助けになれば幸いである．

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(脇谷滋之)

関節軟骨損傷の治療

－最新の知見－

The treatment of articular cartilage injury－recent studies－

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Key words　▶関節軟骨損傷(articular cartilage injury)　▶外傷後変形性関節症(post-traumatic osteoarthritis)　▶組織修復過程(tissue repair process)

はじめに

日常診療における膝関節軟骨損傷を考えた場合，離断性骨軟骨炎などの単独損傷のみならず，前十字靱帯(anterior cruciate ligament；ACL)損傷や膝蓋骨脱臼に合併したものに遭遇することも多い。靱帯再建術などの手術療法が行われた後も，軟骨損傷に起因する変形性関節症(osteoarthritis；OA)の進行に対し長期的な経過観察が必要である。近年，画像診断技術の向上[1]およびバイオマーカーの研究[2]により関節軟骨損傷あるいは初期OAの評価が可能になってきた。さらに，関節鏡視下手術の向上やスポーツ競技人口の増加を背景に，外傷後OAに対する治療介入としての関節軟骨損傷治療に期待が高まっている[3]。関節軟骨損傷に対してはさまざまな治療法が報告されてきているが，現状で行われている治療法にはそれぞれ限界がある。このことは主に関節軟骨が細胞外基質に富む無血管組織であり組織修復過程における細胞や栄養の供給に限界があるためである。さらに修復組織の組成は局所の力学環境にも影響される。軟骨修復過程におけるこれらの生物学的反応を促進するためにさまざまな治療が考案されているが，それらは費用対効果を含め従来法との長期的な優位性を示すことが求められる。また臨床の場では日本国内と欧米を中心とする海外とで薬事規制や社会保険制度などの医療事情が異なるため，どの段階のものを最新の知見とするかは難しい問題である。本稿では現状の治療を概説し，国内で開発中の治療法を中心にいくつかの知見を述べることとする。

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現状における関節軟骨損傷の治療

軟骨損傷に対する現状の一般的な治療法は，主に骨髄刺激法[4]や骨軟骨柱移植[5]が挙げられる。さらに比較的新しい治療法として自家培養軟骨細胞移植術(autologous chondrocyte implantation；ACI，製品名：ジャック®[ジャパン・ティッシュ・エンジニアリング社：2013年4月よりわが国で保険承認])がある[6]。同種の骨軟骨柱移植や細胞移植の臨床応用はアメリカでは行われているが[7]，わが国では困難である。

各治療法は病変部の大きさや程度によって選択される。骨髄刺激法は主に組織修復に寄与する細胞動員を促す目的で行われ，十分な治療効果が得られる範囲は通常2～2.5cm^{2}以下の病変といわれている[8]。骨軟骨柱移植は，力学的にすでに成熟している組織を用いるため，関節軟骨の機能を考えれば有効な治療法である。しかしながら，病変部に相応の健常軟骨組織を採取する必要から治療対象となる損傷の広さには制約があり，4cm^{2}まで

の病変がよい適応とされている[8]。ACI(ジャック®)は骨軟骨柱移植術に比べ比較的少ない侵襲で健常軟骨組織を採取し自家軟骨細胞を体外で増殖させるため，広範囲の病変に対応可能である。適応は4cm^{2}以上の軟骨欠損であり一般には約10cm^{2}までの病変部に対して有効といわれている[8]。

より広範囲の病変への対応にはさらに十分量の細胞数確保のため，増殖後も軟骨分化を誘導できる前駆細胞が望ましく，現在ヒトで応用されているものでは骨髄細胞と滑膜細胞が挙げられる。広範囲の軟骨損傷に対して細胞治療による関節軟骨修復の有効性は明らかであるが，現状では施設の管理・維持に関してインフラ整備へ向けた努力が今後も継続して行われる必要があり，一方では細胞を用いない(セルフリー)治療法の開発も進んでいる。

細胞源と治療法

1 骨髄細胞

骨髄細胞は軟骨前駆細胞として早くから注目されており，自身の軟骨細胞分化能とは別に液性因子分泌による組織修復促進効果も期待されている。また，外来での局所麻酔でも採取が可能であり細胞獲得のための酵素処理などが不要なため，比較的簡便に利用可能なことから，臨床応用に適していると考えられる。1998年，脇谷ら[9]によって世界に先駆けて骨髄細胞を利用した軟骨修復治療の有効性が報告され，今後も継続した検証が必要である。安全面に関しては約11年間に行った41例45膝では少なくとも局所の感染も腫瘍形成も認められていない[10]。

さらに手術侵襲を低減することを目的とし，関節鏡視下の骨髄刺激法に関節内注射による自己骨髄細胞移植を併用した治療法の臨床研究を，西日本を中心とする多施設共同非盲検ランダム化並行群間比較試験として行っている。この方法の有効性が明らかになれば，世界で広く普及する汎用性の高い低侵襲関節軟骨修復治療の開発につながる可能性があると考えている。

また，これらの治療法では利用する骨髄細胞は未分化な状態であり，移植時にはコラーゲンやヒアルロン酸などのスキャフォールドを必要としている。佐藤，高木ら[11]は骨髄細胞からスキャフォールドを用いずに*in vitro*で軟骨様組織を開発することに成功し，実用化へ向けてさまざまな改良を加えている。この再生組織を用いることで，ある程度軟骨へ分化した細胞と細胞由来のマトリックスを同時に欠損部に移植することが可能なため，限局性の軟骨損傷に対して高い治療効果が期待できると考えている。この再生組織がどれだけ広範囲の病変まで対応可能か，作製に要する細胞数確保と再生組織の力学特性について今後の検討課題と考えられる。

2 滑膜細胞

骨髄細胞と同様，滑膜細胞も軟骨修復に応用可能な間葉系細胞として研究されている[12]。関矢らは*in vitro*の実験系で滑膜細胞が増殖能と軟骨分化能において骨髄細胞より優れていることを証明した。彼らは現在，半月板修復に対して滑膜細胞を用いた治療法の開発を積極的に行っている。

3 その他の細胞

体制幹細胞としてはほかにも脂肪由来細胞などが研究されている。またiPS細胞を使った骨軟骨再生の研究も行われている。これらの細胞は，その獲得方法に利点があることから将来的に臨床に利用される可能性が高いと考えられている。

4 セルフリー関節軟骨修復

一方，これら細胞を用いずに，内在性の細胞に働きかけることで組織修復を促進する方法も検討されている。組織修復過程の炎症反応に着目した多血小板血漿(platelet-rich plasma；PRP)やサイトカイン，ケモカインなどを利用した治療法についての報告や[3]，創傷治癒を促進するための高分子化合物(ポリマー)の利用について海外からの報告が散見される[13]。現状においてわが国での利用は困難であるが，今後実用化へ向けた努力がなされるものと考えられる[14]。

ただし，これまでのさまざまな研究にもかかわらず，関節軟骨修復に積極的に関与する細胞に関してはいまだ特定できておらず，これらの治療法で標的となっている細胞の治癒能力がどれだけ誘導できるかは不明なままである。さらにPRPに関しては比較的簡便に利用可能であり有効性も報告されているが、多種類の因子を含んでいるため、

安全性の観点からも臨床利用には作用機序解明を含めた十分な検討が必要と考えられる。現状の軟骨欠損治療と同様，セルフリーで行われる治療法についても，その適応と限界を明らかにし，必要であれば利用可能な細胞との併用を視野に入れることで，より洗練された関節軟骨損傷治療に発展するものと考えられる。

さいごに

外傷などで生じた比較的軽度の軟骨損傷に対しては，OA進行が懸念されるものの短期的には問題となることが少なく，一般的には関節鏡視下手術時でもあまり積極的な治療は行っていないのが現状と考えられる。自然経過で進行するOAとは異なり，これら外傷後のOA進行に対しては治療介入の機会があるため，今後利用可能になる治療法によって対象となる症例の拡大も予想される。各治療法の適応について十分な検討とともに，長期的な優位性についても十分な検証を行うことが今後も継続した課題と考えられる。

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